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Evaluation of mono- and mixed diets as food for intensive *Artemia* culture

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Abstract

In order to alleviate the problems encountered when using live algae or rice bran as *Artemia* food in intensive culturing, different alternative types of brine shrimp diets have been evaluated. Using a high-density flow-through recirculation culture system, a Single Cell Protein yeast and mixed diets, consisting of this yeast and micronized waste products from agricultural crops, were selected as suitable *Artemia* feeds.

Production yields after 2 weeks culturing varied from 2 to 5 kg live weight brine shrimp biomass per culture tank of 300 l. The mixed diets corn/soybean, corn/wheat husks, and corn/yeast were found to be suitable or even better alternatives for the rice bran diet which is commonly used for feeding *Artemia*.

Introduction

Although the cheapest source of brine shrimp biomass is from semi-natural biotopes or man-managed ponds, *Artemia* produced in intensive culture systems is becoming more attractive, especially in climates that are unsuitable for outdoor production and when quality control is critical (Lavens *et al.*, 1985). Since *Artemia* is a non-selective, obligate particle feeder at least with respect to the fulfillment of its requirements for carbohydrates and proteins (Barker-Jörgensen, 1966 ; D'Agostino, 1980), most of the high-density culturing techniques rely on cheap agricultural by-products instead of live algae in order to cut feed costs. Critical in the selection of a suitable diet for intensive culturing are its particle size which should be less than 50 µm (Dobbeleir *et al.*, 1980), its nutritional value, and its digestibility which may be influenced by the colonizing microflora (D'Agostino, 1980 ; Douillet, 1987), and its solubility which should be minimal in order to avoid quality deterioration of the culture water.

Rice bran has been reported to be a cheap and suitable feed source for intensive *Artemia* culture (Sorgeloos *et al.*, 1979 ; Brisset *et al.*, 1982 ; Lavens and Sorgeloos, 1984 ; Platon and Zahradnik, 1987). However, culture success depends upon the batch of rice bran used : *i.e.* the composition of the product may fluctuate according to origin, harvest, processing, etc., and, moreover, may be contaminated with pesticides which are used as a storage treatment (Dobbeleir *et al.*, 1980).

Therefore this study has been conducted to evaluate possible alternatives which are more consistent and reliable in composition, have a better micronization capability, and finally yield

high biomass production of preadult or adult brine shrimp. Special attention has been attributed to the potential use of Single Cell Proteins (SCP) because they have a far more complete nutritional composition, are relatively inexpensive, do not require additional grinding, and ensure a more optimal physical performance of the particles in the culture medium.

Materials and methods

General culturing conditions

Great Salt Lake *Artemia franciscana* (Sanders Brine Shrimp Co., lot 185-O) were hatched under standard conditions (Sorgeloos *et al.*, 1983) for 24 h at 25 °C. After separation and washing from cyst residues the instar I nauplii were counted and inoculated into the culture tanks at a density of 10 000 larvae/l. In the second set of experiments the SCP mono-diet was evaluated at different densities, respectively 5 000, 10 000, and 15 000 nauplii/l.

The 300 l culture tanks are part of a flow-through recirculating system which has been described by Lavens *et al.* (1985) (Fig. 1). Natural seawater originating from the Scheldt-estuary and to which crude seasalt and 1 g/l $NaHCO_3$ is added to obtain a salinity of 50 ‰ and a pH of 8 flows continuously into the culture vessels at varying flow rates ; *i.e.* depending on the growth stage of the brine shrimp water retention times are adjusted from 4 h to 1 h (Table I). Aeration by means of a central aeration collar is adjusted regularly to keep oxygen levels around 4 ppm.

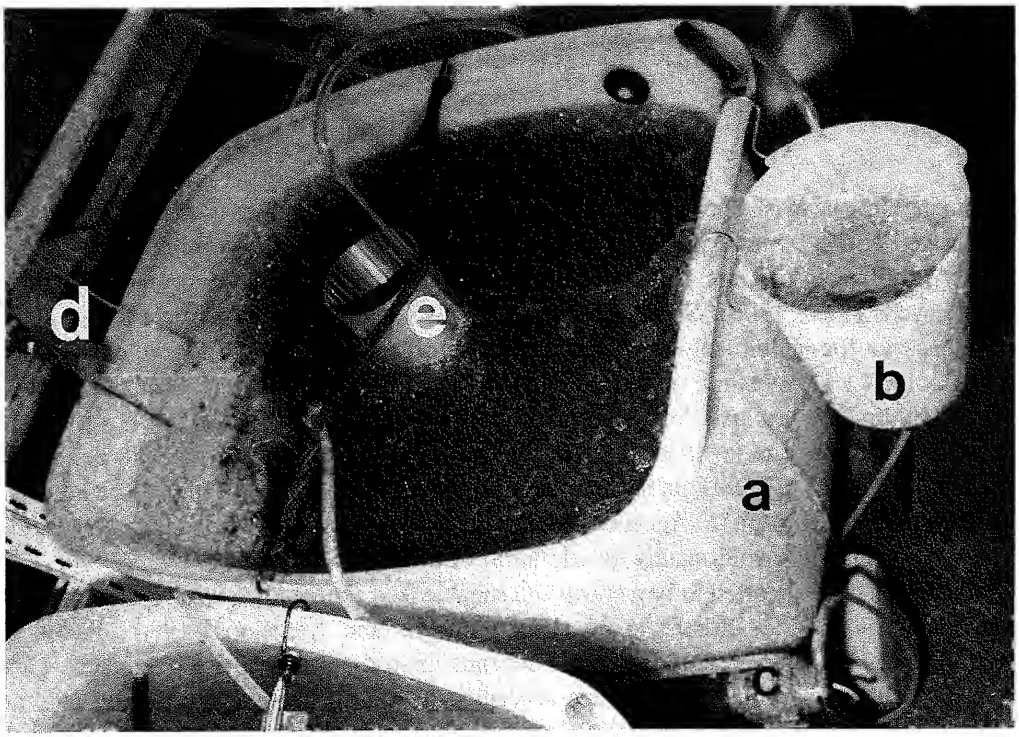

Fig. 1. Overview of the *Artemia* culture unit of the closed flow-through system for brine shrimp biomass production. (a) 300 l culture tank ; (b) feed tank ; (c) feed pump ; (d) water intake ; (e) *Artemia* retaining filter, and water outlet.

TABLE I

Culture water renewal in relation to the growth stage of *Artemia* and the slit opening of the welded-wedge screen filter, as used under optimal culturing conditions

Culture day	Mesh size of filter (µm)	Flow rate (l/h)	Retention time in culture tank (h)
1 - 2	150	80	4
3 - 4	200	100	3
5 - 7	250	150	2
8 - 10	300	150	2
10 - 12	350	200	1.5
12 - 14	450	300	1

FOOD

The different feed components and their chemical composition used in this study are listed in Table II. All agricultural by-products were micronized and supplied by Artemia Systems NV, Belgium ; the SCP-yeast needed no extra processing. Only the yeast has been evaluated as mono-diet. The five mixed diets tested here all contain the corn waste in equal ratios, except for the soybean diet that consists of only 20 % corn waste (Table III).

FOOD PREPARATION AND DISTRIBUTION

In order to avoid bacterial degradation, fresh diet suspensions were made up daily by mixing the suspension of dry feed and saturated NaCl brine in a kitchen blender. Optimal feeding conditions were created by adding food suspension on a semi-continuous basis, *i.e.* food pumps were controlled by electronic time clocks which were adjusted daily to maintain a culture medium transparency of 20-25 cm. Only in the second set of experiments different transparency levels were applied (Table IV).

DATA COLLECTION

Individual length and survival rates were recorded daily by sub-sampling the culture tanks : *i.e.* three samples of 25 ml were taken and fixated with lugol solution. The average length of 30 brine shrimp measured from the top of the head to the base of the caudal furca was determined using a dissecting microscope equipped with a drawing mirror. Biomass production was estimated by wet weight analysis of the *Artemia* collected on a small sieve from a 1 l sample. Food conversion efficiency was calculated by dividing the total amount of feed added until that day by the wet-weight biomass amount of that moment.

Results

In the first set of experiments a high *Artemia* biomass production was achieved after 14 days culturing with the diets partially consisting of corn and mixed with soybean product (diet A/E), wheat product (A/D), or yeast (A/F) (Table III, Fig. 2). If the yeast series would not have faced a high mortality starting from day 10 onwards (Fig. 3), much higher biomass production-levels

TABLE II

Average chemical composition of the various feed components used in the *Artemia* diets

Feed origin (code)	Description of feed	Crude protein (%)	Crude fat (%)	Carbohydrates (%)	Ash (%)
Corn (A)	Mixture of waste products from corn industry	62	6	20-27 + 3-10 % fibers	2
Rice (B)	Waste product from polishing rice	11-12	11-13	25-33 + 3-13 % fibers	8-12
Wheat (C)	Wheat germ product	25	8	25 + 3 % fibers	4
Wheat (D)	By-product from wheat industry ; contains mostly husks	14	3	20 + 11 % fibers	6
Soybean (E)	Waste product from soybean industry ; contains small amounts of soybeans	9	1	86 (mainly fibers from cellulosic type)	4
SCP : yeast (F)	Commercially available dry *Kluyveromyces* spp. yeast	> 48	6-8	28-33 (mainly mannanes and glucanes)	7-9

would have been obtained (*i.e.* more than 20 kg). This can be extrapolated from the large biomass increase during the 1st week which was twice as good as with the other feeds. The wheat diet (A/C) and the yeast mono-diet (F) did not perform well in terms of biomass yields after 14 days culturing. As compared to the other diets, the latter two happen to contain high protein levels (> 40 %) and only small amounts of fibers (Table II). Although good survival and relatively good length increase were obtained on diet A/C, poor biomass production prevailed. This indicates that the average length is not always a good estimate of the brine shrimp's growth.

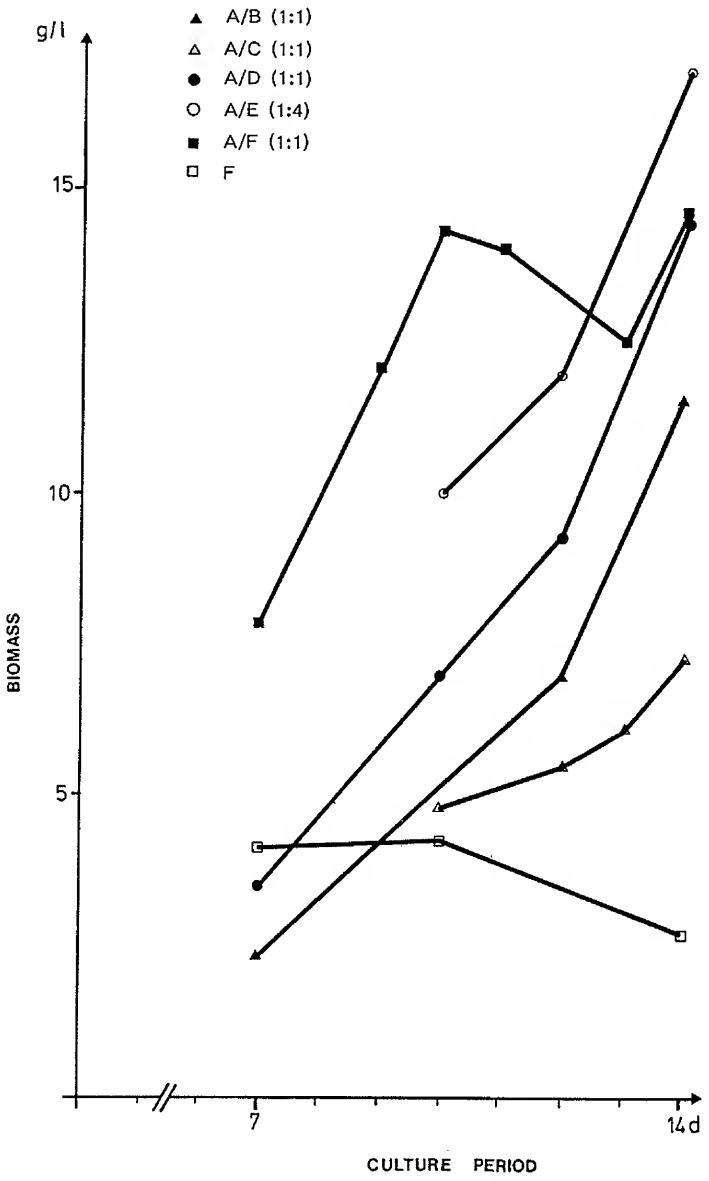

FIG. 2. Biomass production of Great Salt Lake *Artemia* in a flow-through recirculation system, using different mono- and mixed diets as food.

TABLE III

Production data of Great Salt Lake *Artemia franciscana* cultured on various diets during 14 days in 300 l culture tanks (D7, D10, D14 = results after 7, 10 resp. 14 days culturing)

Diet composition[1]	Survival (%)			Length (mm)			Biomass production (g wet wt/l)			Conversion efficiency (g dry wt/g wet wt)		
	D7	D10	D14	D7	D10	D14	D7	D10	D14	D7	D10	D14
A/B 1:1	93	74	73	1.8	2.9	3.5	2.3	–	11.6	1.60	–	0.65
A/C 1:1	71	70	70	2.2	3.5	5.6	–	4.8	7.2	–	0.84	1.04
A/D 1:1	85	86	71	2.2	3.8	4.9	3.5	7.0	14.5	1.30	1.10	0.80
A/E 1:4	65	58	57	3.0	4.8	5.8	–	10.0	17.0	–	0.80	0.75
A/F 1:1	80	61	35	3.6	5.9	7.7	7.9	14.3	14.7	0.51	0.50	0.82
F	76	56	15	2.1	3.6	5.1	4.1	4.3	2.6	0.48	0.58	2.05

[1] Abbreviations used are explained in Table II.

TABLE IV

Production data of Great Salt Lake *Artemia franciscana* cultured under different conditions and fed with yeast (D7, D10, D14 = results after 7, 10 resp. 14 days culturing)

Specific culture conditions		Survival (%)			Length (mm)			Biomass production (g wet wt/l)			Conversion efficiency (g dry wt/g wet wt)		
Density (*Artemia*/l)	Transparency (cm)	D7	D10	D14	D7	D10	D14	D7	D10	D14	D7	D10	D14
10 000	20-25	76	56	15	2.1	3.6	5.1	4.1	4.3	2.6	0.48	0.58	2.05
10 000	30-35	63	69	19	2.8	3.8	4.3	3.8	7.7	5.0	0.52	0.60	0.98
15 000	40-45	75	65	38	2.7	3.6	3.8	3.5	6.5	6.4	0.46	0.54	0.83
5 000	40-45	87	87	63	2.9	4.3	4.3	2.0	3.7	6.0	0.55	0.57	0.52

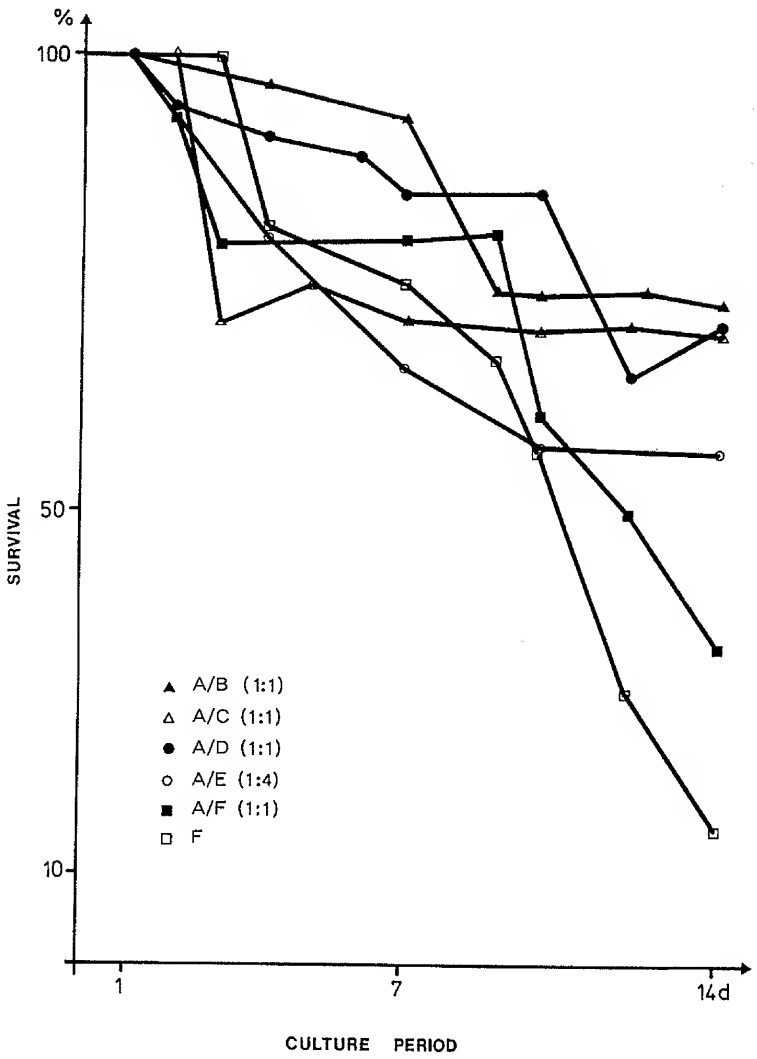

FIG. 3. Survival rates of Great Salt Lake *Artemia* cultured in 300 l flow-through culture tanks for 14 days on different mono- and mixed diets.

Food conversion efficiency at day 14 is highly correlated (r = -0.9003) with final biomass production ; however, an exception should be made for the rice bran mixed-diet. This means that under optimal production circumstances, *i.e.* when yields of 15 kg adult *Artemia*/m^3 of culture medium are attained, approximately 12 kg of a 'good' food is required. The data also show a large difference until day 10 between the micronized diets on the one hand, and the mono- and mixed SCP diets on the other hand. Conversion efficiencies for the latter experiments were already high during early *Artemia* development (± 0.5) while for the other diets only conversions above 1.0 were obtained during the first part of the culture period.

Survival rates remained high (> 60 %) throughout the culture period of 14 days for all micronized diets tested, but declined rapidly for both yeast diets (Fig. 3). Both A/E and A/C showed mortalities from the start onwards but kept constant survival rates afterwards. Growth rates, expressed as increase in length, varied considerably among the different trials (1.8 to 3.6 mm) already after 7 days of culturing. A fast growth during the naupliar stages resulted always in a good growth performance during the rest of the culture period. No relationship between mortality (= lower population density) and growth rate could be established, which supports the idea that the food concentration is not limiting and that *Artemia* densities up to 10 000/l in the present flow-through culture system do not cause a stress situation for the *Artemia*.

The second series of experiments was set up to verify if no overfeeding had occurred when using yeast ; *i.e.* because of its physical nature yeast ensures a better food availability for the *Artemia* as it consists of individual cells of appropriate size and buoyancy. Therefore, lower transparency levels of the culture medium were maintained and the larval density varied from 5 000 to 15 000 *Artemia*/l. Significant effects especially on survival and biomass production were recorded for the lowest feeding regimes (Table IV). However, the total biomass output remained rather low because of the stop in growth and a high mortality from day 10 onwards.

Discussion

D'Agostino (1980) indicated that the success of *Artemia* cultures fed agricultural by-products is strictly dependent on the instantaneous establishment of a microflora as a supplement to the nutrient-deficient diets. Also Douillet (1987) hypothesized that stable *Artemia* cultures are only sustained when a balance between favorable and unfavorable microbial species is established. Using flow-through culture systems as applied here might make the dietary requirements less or more critical since the impact of favorable/unfavorable bacteria is reduced because part of the microflora is continuously drained off with the effluent. The variable results which are often obtained with these flow-through recirculation systems, even under so-called standard conditions, may be due to specific bacterial developments in individual culture tanks. For this reason the results obtained with these experiments only provide tendencies and should not be statistically interpreted.

Present literature on the nutritional requirements of *Artemia* is very scarce. D'Agostino (1980) suggested that brine shrimp require more carbohydrates than proteins and little if any fatty acids. According to Hanaoka (1973) and Sick (1976) growth rates of *Artemia* are positively correlated with the amount of crude protein in the diet. Levels above 28 % protein will, however, negatively affect growth by deteriorating the water quality of the culture medium (Hanaoka, 1973). Our results suggest that protein content as a sole factor is not of major importance in obtaining high yields of adult *Artemia* biomass. The diets A/E and A/F contain 34 %, respectively 55 % crude protein and both gave maximal yields, *i.e.* 15 g wet wt/l. Carbohydrates of fibrous nature (such as the soybean waste) apparently ensure good growth (results with diet A/E *versus* diets A/C and F), either as a dietary ingredient or as a bacterial substrate.

The physical characteristics of potential *Artemia* feeds need also to be taken into consideration, *e.g.* micronized products have the tendency to easily form aggregates in water, and therefore can no longer be ingested by the *Artemia* and may clogg the *Artemia* retaining filter. Diets, on the contrary, that enhance fast *Artemia* growth especially in the early juvenile stages, will allow a

faster change to larger mesh filters and to work at shorter retention times. In this respect, Single Cell Proteins might offer interesting opportunities. Yeasts for example are available as individual cells of appropriate size that do not pollute the water. As their nutritive substances are isolated in a rigid cell wall, the growth of microflora is also reduced. This better physical availability of a yeast diet probably allows to work at lower particle loads or water transparencies (Table IV).

Shimaya *et al.* (1967) fed different types of marine yeast to *Artemia* and recorded a growth to 9 mm in 14 days culturing, and survival rates varying from 50 to 90 %. Nimmannit and Assawamunkong (1985) also used a marine yeast but obtained good growth only until day 4. Using the *Kluyveromyces* yeast as mono-diet we also observed good survival and growth rates only during the first week of culturing. This might be due to a deficiency or imbalance of nutrients as is hypothesized by Hirayama and Funamoto (1983), Hirayama (1987) for *Brachionus*, and Urban and Langdon (1984) for *Crassostrea* cultures. The fact that the mixed diet yeast/corn waste gave the best results (only up to the pre-adult stage after 10 days culturing) provides further evidence for the deficiency hypothesis. Similarly, Robin *et al.* (1981) showed that a 50 % substitution of a *Spirulina* diet by brewers' yeast resulted in a significantly better survival of the SFB *Artemia* after 6 days of culturing.

From this study we can conclude that depending on the local availability and prices, various substitutes for rice bran can be considered for the intensive production of *Artemia* in flow-through culture conditions. These *Artemia* can be used as an acceptable food source for various predators (see review by Léger *et al.*, 1986). However, the nutritional composition of the *Artemia* especially with regard to essential fatty acids (*e.g.* 20:5ω3 and 22:6ω3) is expected to be very low. This deficiency can be remedied by application of bioencapsulation techniques (Sakamoto *et al.*, 1982 ; Léger *et al.*, 1987). With the new enrichment procedures, nutritional compositions can be modified in a few hours time, ensuring a nutritionally excellent prey for aquaculture species (Léger, pers. commun.).

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Literature cited

BARKER-JÖRGENSEN C. 1966. Feeding. p. 69-113. In : Marine biology III. Proc. 3rd Int. Interdisciplinary Conf. Edmondson W. T. (Ed.). New York Academy of Sciences, Interdisciplinary Communications Program, New York, USA.

BRISSET P. J., D. VERSICHELE, E. BOSSUYT, L. DE RUYCK, and P. SORGELOOS. 1982. High density flow-through culturing of brine shrimp *Artemia* on inert feeds. Preliminary results with a modified culture system. Aquacult. Eng. 1(2):115-119.

D'AGOSTINO A. S. 1980. The vital requirements of *Artemia*, physiology and nutrition. p. 55-82. In : The brine shrimp *Artemia*. Vol. 2. Physiology, Biochemistry, Molecular biology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 636 p.

DOBBELEIR J., N. ADAM, E. BOSSUYT, E. BRUGGEMAN, and P. SORGELOOS. 1980. New aspects of the use of inert diets for high density culturing of brine shrimp. p. 165-174. In : The brine shrimp *Artemia*. Vol. 3.

Ecology, Culturing, Use in aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 428 p.

DOUILLET P. 1987. Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

HANAOKA H. 1973. Cultivation of three species of pelagic micro-crustacean plankton. Bull. Plankton Soc. Jap. 20(1):19-29 (in Japanese, English abstract).

HIRAYAMA K. and H. FUNAMOTO. 1983. Supplementary effect of several nutrients on nutritive deficiency of baker's yeast for population growth of the rotifer *Brachionus plicatilis*. Bull. Jap. Soc. Sci. Fish. 49(4):501-510.

HIRAYAMA K. 1987. A consideration of why mass culture of the rotifer *Brachionus plicatilis* with baker's yeast is unstable. Hydrobiologia 147:269-270.

LAVENS P., P. BAERT, A. DE MEULEMEESTER, E. VAN BALLAER, and P. SORGELOOS. 1985. New developments in the high density flow-through culturing of brine shrimp *Artemia*. J. World Maricult. Soc. 16:498-508.

LAVENS P. and P. SORGELOOS. 1984. Controlled production of *Artemia* cysts under standard conditions in a recirculating culture system. Aquacult. Eng. 3:221-235.

LÉGER Ph., D. A. BENGTSON, K. L. SIMPSON, and P. SORGELOOS. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Annu. Rev. 24:521-623.

LÉGER Ph., E. NAESSENS-FOUCQUAERT, and P. SORGELOOS. 1987. International Study on *Artemia*. XXXV. Techniques for manipulation of the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

NIMMANNIT S. and S. ASSAWAMUNKONG. 1985. Study on yeast as feed for marine organisms (brine shrimps). In : Proc. Living Aquatic Resources. Chulalongkorn Univ., March 7-8, 1985, Thailand.

PLATON R. R. and J. W. ZAHRADNIK. 1987. Scale-up studies on the culture of brine shrimp *Artemia* fed with rice bran. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

ROBIN J. H., F. J. GATESOUPE, and R. RICARDEZ. 1981. Production of brine shrimp (*Artemia salina*) using mixed diets : consequences on rearing of sea bass larvae (*Dicentrarchus labrax*). J. World Maricult. Soc. 12(2):119-120.

SAKAMOTO M., D. L. HOLLAND, and D. A. JONES. 1982. Modification of nutritional composition of *Artemia* by incorporation of polyunsaturated fatty acids using micro-encapsulated diets. Aquaculture 28:311-320.

SHIMAYA M., A. KANAZAWA, and K. KASHIWADA. 1967. Studies on the utilization of marine yeast. I. Culture of *Artemia* and *Daphnia* by marine yeast. Mem. Fac. Fish. Kagoshima Univ. 16:34-39 (in Japanese).

SICK L. V. 1976. Nutritional effect of live species of marine algae on the growth, development, and survival of the brine shrimp *Artemia salina*. Mar. Biol. 35:69-78.

SORGELOOS P., E. BOSSUYT, E. BRUGGEMAN, A. COOREMAN, J. DOBBELEIR, P. VANHAECKE, and D. VERSICHELE. 1979. Progress report on the research activities of the Artemia Reference Center. ICES-CM ; F54:206-214.

SORGELOOS P., E. BOSSUYT, P. LAVENS, P. LÉGER, P. VANHAECKE, and D. VERSICHELE. 1983. The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. p. 71-96. In : CRC handbook of mariculture. Vol. I. Crustacean aquaculture. McVey J. (Ed.). CRC Press, Boca Raton, Florida, USA. 442 p.

URBAN E., Jr. and C. LANGDON. 1984. Reduction in costs of diets for the american oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. Aquaculture 38:277-291.